

Synthesis of Anti-Ageing Reagent: Sulfo Disaccharide Co-Working with Anti-Ageing Gene

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Short Communication

Anti-ageing reagents: Sulfo disaccharides co-work with klotho was prepared by organic synthesis. By comparing of these compounds by easiness of getting starting compounds, reaction steps, easiness of reactions, easiness of purification, activities, easiness of handling and solubility. p-methoxyphenyl-3-sulfo- β -D-glucuronosyl-(1-4)-2-acetoamino-3-deoxy-glucopyranoside 9294 is selected as best compound for research on anti-ageing.

Nabeshima found Klotho (anti-ageing gene) [1]. Since then many reports on klotho [2-29] are published.

Nabeshima found a sulfo disaccharide from mouse liver cell. This saccharide bind with Klotho I synthesized five sulfo disaccharide [30]. At the second paper [31], I have proposed the hypothesis that klotho make disaccharide from glucuronic acid and glucosamine on site and klotho (anti-ageing gene) co-work with his produced disaccharide (anti-ageing reagent) and provide Ca homeostasis and consequent anti-ageing and long life. This proposed mechanism must be proved by many others persons. I described synthetic procedure in detail for 9279, 9274, 9188 at first paper [30]. The synthesis of these sulfo disaccharide are not easy. I wish to describe synthetic method of 9294, most easily obtainable anti-ageing reagent at this paper.

Anti-ageing reagent: Sulfo disaccharide

To get proof of the hypothesis, we must get easily accessible disaccharide for the research to know how klotho and disaccharide are working. At this paper the easiness of synthesis and easiness of handling of 5 disaccharides were compared. It was found that p-Methoxyphenyl-3-sulfo- β -D-glucuronosyl-(1-4)-2-acetoamino-2-deoxy-glucopyranoside 9294 was as a best compound for this purpose.

We can consider four possible structure A,B,C and D As an example of compounds C, 3-estrone-3-sulfo -glucuronosyl (1-3)-2-acetoamino-2-deoxy- β -D-glucopyranoside 9279 were prepared. As an example of compound D Estrone-3-sulfo-glucuronosyl(1-4)-2-acetoamino-2-deoxy-glucopyranoside 9214, p-methoxyphenyl 3-sulfo-glucuronosyl (1-4)-2-acetoamino-2-deoxy-glucopyranoside 9294 As an example of compound A, p-methoxyphenyl-3-sulfo-glucuronosyl(1-4)-2-acetoamino-2-

deoxy-galactopyranoside 9583 was prepared. The synthesis of p-methoxyphenyl-3-sulfo-glucuronosyl (1-4) -2-acetoamino-2-deoxy-glucopyranoside 9294 was carried out as shown in **Figure 1**.

By the reaction of p-methoxyphenyl-3,6-di-O-benzyl-2-acetoamino-2-deoxy-glucopyranoside with 1-bromo-2,3,4-tri-O-acetyl-D-glucuronate, (1-4) bonded glucoside was obtained. 3,6-di-Benzyl are cleaved by hydrogen reduction. Methyl group and acetoxy groups are cleaved by LiOH hydrolysis. Sulphonation by $\text{SO}_3\text{-TEt}_3$ gave desired p-methoxyphenyl-3-sulfo - glucuronosyl-(1-4)-2-acetoamino-2-deoxy-glucopyranoside 9294.

Structure activity relationship

The binding activities of synthesized compounds, 9214, 9188, 9583, 9279, 9244 were measured. The activities of these 5 compounds were almost same irrespective of (1-4) or (1-3) bond, glucopyranoside or galactopyranoside, p-methoxyphenyl, or estrone, or serine.

Comparison of preparation steps

Estrone-3-sulfo-glucuronosyl (1-3)-(2-acetoamino-2-deoxy-glucopyranoside) 9279 is prepared by 9 steps as shown at **Figure 1** [30].

Serine-3-sulfo-glucuronosyl-(1-3)-2-acetoamino-2-deoxy-glucopyranoside 9244 is prepared by 9 steps as shown at **Figure 2** [30].

3-Estrone 3-sulfo-glucuronosyl-(1-4)-2-acetoamino-2-deoxy-glucopyranoside 9188 is prepared by 8 steps as shown at **Figure 3** [30].

Gln-His-Thr-3-sulfo-glucuronosyl-(1-3)-2-acetoamino-2-deoxy-glucopyranoside 9205 is prepared by 9 steps as shown at **Figure 4** [1]. p-Methoxyphenyl-3-sulfo-glucuronosyl-(1-4)-2-acetoamino-glucopyranoside 9294 is prepared by only 4 steps as shown at Figure 1 of this paper.

Comparison of properties of disaccharides

p-Methoxyphenyl-3-sulfo-glucuronosyl (1-4)-2-acetoamino-2-deoxy-glucopyranoside 9294 is soluble in organic solvent and lithium or sodium salt is soluble in water and easy to purify by chromatography.

Estrone-3-sulfo-glucuronosyl-(1-3)-(2-acetoamino-2-deoxy-glucopyranoside) 9279 and 3-Estrone-O-(β -D-3-sulfo-glucuronosyl)-(1-4)-2-acetoamido-2-deoxy-glucopyranoside 9214 are insoluble in chloroform and water. And purification by chromatography is very difficult.

Conclusion

Considering these facts, p-methoxyphenyl-3-sulfo- β -D-glucuronosyl-(1-4)-2-acetoamino-3-deoxy-glucopyranoside 9294 looks like a best compounds. But this is only best in 5 compounds. By expanding the kind of sugar, by changing or at glucosamine 1-position, discovery of much better anti-ageing reagents will be discovered.

Experimental part

Synthesis of p-methoxyphenyl-3-sulfo-glucuronosyl-(1-4)-(2-acetoamino-2-deoxy-glucopyranoside) 9294 [33-38]

- (1) p-Methoxyphenyl-O-2,3,4-tri-O-acetyl methyl glucuronosyl-(1-4)-2-acetoamino-2-deoxy-3,6-di-O-benzyl-glucopyranoside 9285 p-Methoxyphenyl-2-acetoamido-3,6-di-O-benzyl-2-deoxy- β -D-glucopyranoside (obtained by the acetylation of p-methoxyphenyl-2-amino-3,6-di-O-benzyl-2-deoxy- β -D-glucopyranoside(Tokyo Kasei M1616)) 420 mg (0.828 mmole) was dissolved in CH_2Cl_2 6 ml and,

silvertriflate 348 mg(1.65 mmole), Molecular sieve 3A 0.7g under dry nitrogen atmosphere cooled at -40°C A solution of 1-bromo-2,3,4-tri-O-acetyl-D-glucuronate [38] 460 mg (1.28 mmole) and collidine 138 mg in 5 ml dichloromethane was added drop wise to the cooled solution. After 2 hours, warmed to room temperature over night with stirring. After addition of dichloromethane 10 ml, molecular sieve was filtered. Washing of the filtrate with 3% hydrochloric acid, saturated sodium bicarbonate, and water. The solution was dried and evaporated. The product was purified on silica gel with ethyl acetate and hexane 1:1.9285 647 mg (95%) was obtained.

- (2) p-Methoxyphenyl-(β -D-2,3,4-triacetyl-glucuronosyl)-(1-3)-2-acetoamino-2-deoxy-glucopyranoside 9290, 9285 100.9 mg 0.122 mmol Pd/C 53 mg is dissolved in CH_3OH . 5 ml stirred under H_2 at room temperature overnight. Pd/C is filtered and the reaction mixture is evaporate to get 9290 50.6 mg(64.5%).

- (3) p-Methoxyphenyl- β -D-glucuronosyl-(1-4)-2-acetoamino-2-deoxy-glucopyranoside 9292.

9290, 9.9 mg(0.0148 mmol) is dissolved in LiOH solution (LiOH 1.75 M aqueous solution 16.5 μl and THF 100 μl). After 5 minutes, the reaction mixture is evaporated under vacuum to get residue 10 mg of 9292.

Mass analysis showed 503.197. Theoretical exact mass is 503.1639.

- (4) 3-Sulfo p-methoxyphenyl- β -D-glucuronosyl-(1-4)-2-acetoamino-2-deoxy-glucopyranoside 9294.

The solution of 9292, 5 mg (0.00856 mmol) and $\text{SO}_3\text{-NEt}_3$ 4.65 mg [39] in dimethylformamide 50 μl are stirred for 2 hours and evaporated under vacuum. This process was repeated 3 times to get 6 mg 9294.

9294 activated the binding of klotho and FGF 23.

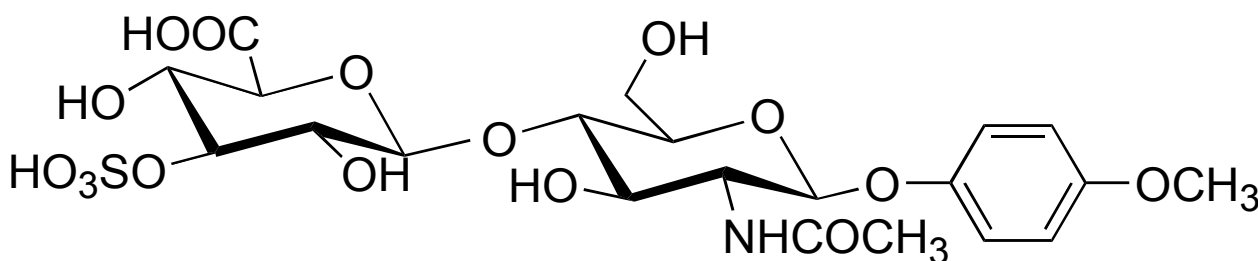


Figure 1 p-methoxyphenyl-3-sulfo- β -D-glucuronosyl-(1-4)-2-acetoamino-3-deoxy-glucopyranoside 9294.

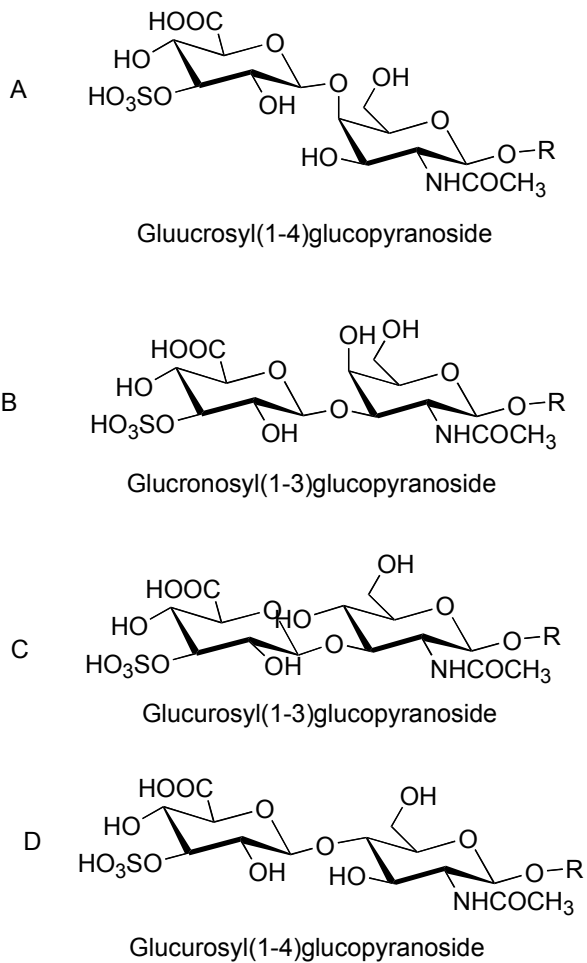


Figure 2 (A) Glucosyl(1-4)glucopyranoside, (B) Glucronosyl(1-3)glucopyranoside, (C) Glucosyl(1-3)glucopyranoside, (D) Glucosyl(1-4)glucopyranoside.

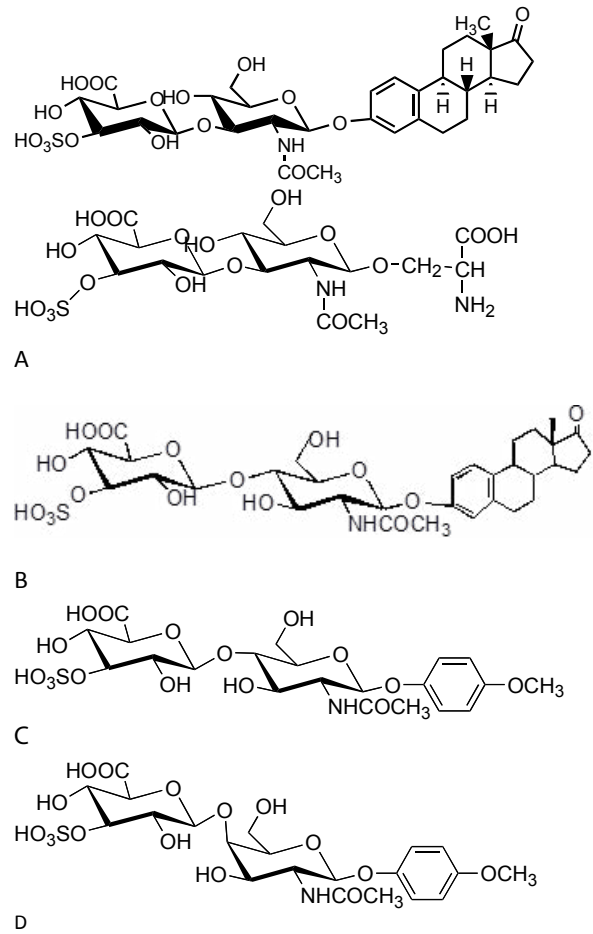
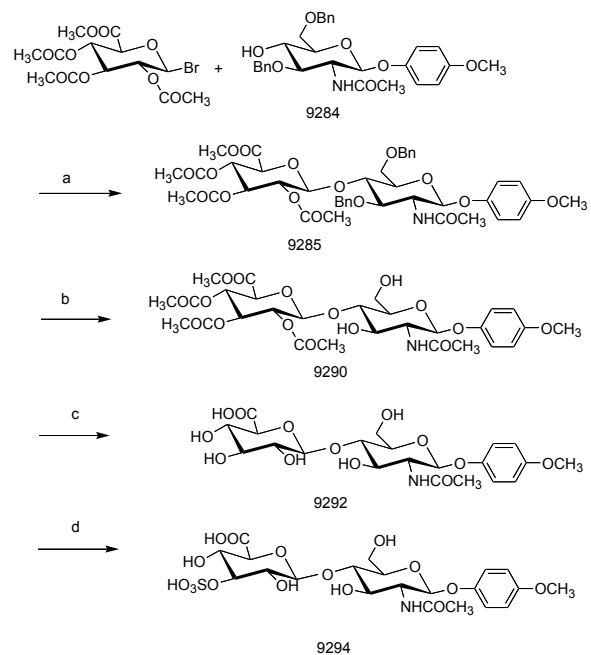


Figure 3 (A) 3-estrone-3-sulfo-β-D-glucuronosyl-(1-3)-2-acetamido-2-deoxy-β-D-glucopyranoside 9279, (B) Serine-3-sulfo-glucuronosyl-(1-3)-2-acetamido-2-deoxyglucopyranoside 9244, (C) 3-Estrone-O-(β-D-3-sulfo-glucuronosyl)-(1-4)-2-acetamid-2-deoxyglucopyranoside 9214, (D) p-methoxyphenyl-3-sulfo-glucuronosyl-(1-4)-(2-acetamido-2-deoxyglucopyranoside) 9294.



a: AgOTf, b: H₂ Pd/C, c: LiOH, d: SO₃ NEt₃

Figure 4 Synthesis of p-methoxyphenyl-3-sulfo-β-D-glucuronosyl (1-4)-2-acetoamino-2-deoxy-glucopyranoside 9294.

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